

**SURVEY OF *FUSARIUM* SPECIES
IN AN ARID ENVIRONMENT OF BAHRAIN.
IV. PREVALENCE OF *FUSARIUM* SPECIES
IN VARIOUS SOIL GROUPS USING
SEVERAL ISOLATION TECHNIQUES**

QAHER A. MANDEEL

Department of Biology, College of Science, University of Bahrain,
P.O. Box 32038, Isa Town, State of Bahrain

ABSTRACT — Three isolation techniques, namely soil dilution, soil plating and soil baiting were used to recover six *Fusarium* species from various soil groups in the arid environment of Bahrain. A total of 428 isolates were recovered using the above techniques. Soil samples analyzed were generally poor in organic matter, slightly alkaline, highly saline and low in total soluble salts. Maximum isolate recovery and species spectrum were mainly recorded from soils of regosols, characterized by low salinity levels ($660 \mu\text{S cm}^{-1}$). Of species encountered, *Fusarium solani*, *F. equiseti* and *F. compactum* were dominant and frequent in all soil groups. Species prevalence, distribution and total isolates were greatly influenced by soil salinity, which counteracted other soil parameters. The soil dilution plate yielded the highest population level and species diversity from regosol soils. Significant interaction occurred between the applied technique and species recovery, as determined by chi-square analysis. Results obtained are in favour of the use of multi-isolation techniques and media approach for comparing survey studies of soil-borne *Fusaria* from noncultivated desert environment.

KEY WORDS - Arid environment, *Fusarium*, isolation techniques, soil group, survey.

INTRODUCTION

Bahrain is a small island nation in the Arabian Gulf consisting of an archipelago of 33 islands; these are situated 25 Km off the eastern coast of the Saudi Arabian Peninsula with a total area of about 695 Km^2 (Fig. 1). According to Doornkamp *et al.* (1980), the main island of Bahrain can be divided into five major physiographic zones: coastal lowlands, backslopes, escarpment, interior basin and central plateau and jebels. Most soils are saline, calcareous, gypsiferous and coarse in texture, and as such are closely related both geologically and climatologically to the Arabian Peninsula. Also as in other arid environments, soils are generally of low fertility being poor in organic matter content (<0.05-1.51%) and nutrient level. The water-holding capacity is low and available moisture about 2.6%. Microbial tolerance to such an extreme ecosystem also characterized by high temperatures is critical for their survival.

The genus *Fusarium* is one of the most common member of soil-biotic microflora (Burgess, 1981; Stoner, 1981). Geographic distribution of *Fusaria* in various ecological zones are well documented (Burgess *et al.*, 1988; Kommedhal *et al.*, 1988; Marasas *et al.*, 1988; Jeschke *et al.*, 1990; Burgess & Summerell, 1992; Sanglang *et al.*, 1995). However, the majority of surveys on *Fusarium* diversity have focused on cultivated soils and there have been few studies on noncultivated ones (Booth, 1977). This situation gives the impression the genus is rare in nature or ecologically unimportant in noncultivated soils, despite the fact that known *Fusaria* have been reported from all types of soils (Stoner, 1981).

Noncultivated soils and habitats of *Fusaria* include forests, scrub communities, savannahs, prairies, pastures and other grasslands, deserts, swamps, littorial and coastal zones. Mandeel *et al.* (1995) and Abbas & Mandeel (1995) recently reported on the occurrence of *Fusarium* species in the arid deserts of Bahrain. Diversity and abundance were correlated to soil factors such as organic matter and salinity (Moubasher & Al-Subai, 1987; Khodair *et al.*, 1991) rather than with climatological conditions (Burgess *et al.*, 1988). However, no information is available on the abundance and distribution of *Fusaria* in soil groups of arid deserts. Data on population dynamics, species variability, species inter-and intra-relationships and mechanisms of existence under extreme and stressful environments could provide a better understanding on the native fungus ecosystem.

The purpose of this study is to survey the abundance and distribution of *Fusaria* in various noncultivated soil groups of Bahrain arid desert and compare the effect of three isolation techniques on their recovery.

MATERIALS AND METHODS

Climate

Bahrain, like mainland Arabia, falls in the North African-Euroasian climate province. The main island has a typical Saharo-Arabian climate, characterized by hot, humid summers and mild winters with low annual rainfall. Average monthly rainfall and temperature in the main island is illustrated in Fig 2. According to climate norms obtained from the Civil Aviation Directorate (Bahrain, 1990-1996), the mean annual temperature is 17.3° C, with a recorded June maximum of 47.5° C and a January minimum of 2.8° C. Total annual rainfall range between 0 to 17 mm. The wettest month is January with up to 17 mm, while May to October is have very low rainfall of less than 0.05 mm.

Soils

According to Doornkamp *et al.* (1980) and Abbas & El Oqlah (1992), soils of Bahrain island are formed from Holocene and Pleistocene sedimentary rocks; they could be divided into five major groups (Fig. 1):

(1) Cultivated solonchak: this group is located on the northern coastal lowlands extending from Jurdab in the east, through the coastal area of Tubli Bay, Manama, Diraz, Sar, Hamalah, to Dar Kulaib in the west. Soils are composed of loamy, sandy and clay subgroups. Normally they are characterized by having a high water table. They are usually cultivated with the main crops being date palm and tomatoes.

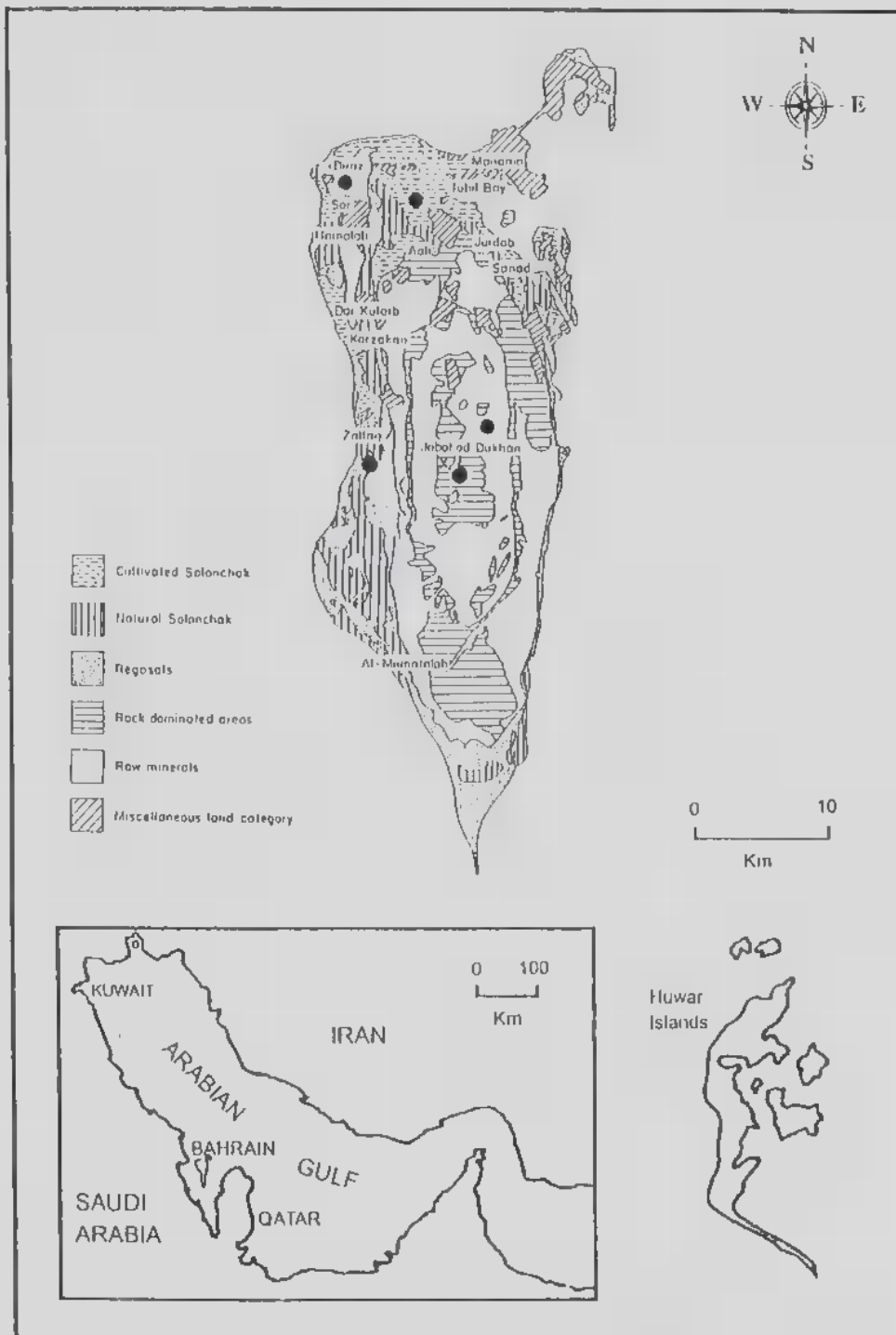


Figure 1. Location map of Bahrain representing the soil groups (extracted from Abbas & El-Oqlah, 1992) where collection of soil samples were made.

(2) Natural solonchak: this group of soils is found between the interior of the northern coastal lowlands and the base of the backslopes, extending from Sanad in the east, through northern Aali, Karzakan and Zallaq, to the Al-Mumatalah in the south-west. Here soils contain gypsiferous solonchak and sabkha subgroups. Natural solonchak soils are usually quartz-gypsiferous sands, salt pans and marine mudflats; they too are characterized by having a high water table.

(3) Regosols: this group of soils is mainly an almost continuous strip in the coastal areas. It is also found between coastal lowlands and backslopes. Two sub-groups are recognized: aeolian sands and recent beach deposits. They are mainly in the form of stable sands, dune sands and beach sands.

(4) Raw mineral soils: raw mineral soils are found in the backslope zone, the escarpment, and in the north-eastern part of the central basin. Three subgroups are recognized: soils of the interior basin, soil detrital fans and stone pavement soils. These soils are usually loamy, sandy or gravely.

(5) Rock dominant areas: these are restricted to the central part of the island. Such areas constitute all the central plateau, some of the basin, the escarpment and some parts of the northern and southern backslopes.

Soil sampling procedures and analysis

Soil samples were collected from five locations, namely As-Sehla, Zaalaq, Duraz, Awali and Sakhir, adequately representing the five soil groups. All locations are within the central and southern part of the main island (Fig. 1). Sampling sites and physiographic zone, soil type, dominant plant communities and respective soil chemical analysis data are listed in Table 1. Each soil group was sampled once during two winter seasons: January 1995 and January 1996.

For each soil group, four composite soil samples, 2 Kg each, were collected, some 20-30 m apart. Each sample consisted of about 10 subsamples (200g), taken with a clean hand-trowel, approximately 4 m apart and from the upper 15 cm of the soil profile. Subsamples were combined, placed in paper bags, labeled, air-dried and stored at 5° C until processed, within one week (Burgess & Summerell, 1992). Although samples were all taken from all soil groups dominated by natural vegetation, care was taken to collect them away from plant canopies or roots to avoid a rhizosphere effect.

For the assays, composite soil samples were thoroughly hand-mixed in plastic bags under sterile conditions and divided in two parts. The first was stored at 5° C, the second was used for chemical analyses. Soil pH and electrical conductivity ($\mu\text{S cm}^{-1}$) were determined in a 1:5 soil:water extract using a JENWAY Water Analyser (Model PW1). Organic matter content (%) was determined by ashing 100 g of air-dried soil in a furnace at 600° C for 1 hr and estimating difference in weight. Total soluble salts (TSS) were measured by mixing 20 g with 100 ml distilled water, filtering and evaporating the filtrate at 105° C. The dry residue was then weighed and the TSS calculated. Table 1 presents the average values of three soil replicates.

Isolation

The second part of the sample was further air-dried, homogenized, crushed to ■ fine powder when necessary and passed through a 0.5 mm soil screen to remove root fragments and other debris (Kreutzer, 1972). Three techniques were used to isolate

Physiographic Zone	Soil Groups				Rock Dominated Areas (RD)
	Cultivated Solonchak (CS)	Natural Solonchak (NS)	Regosols (R)	Raw mineral (RM)	
Location	Coastal lowlands	Coastal lowlands	Coastal lowlands	Central Basin	Central Depression
Soil Subgroup	As-Sekia Loamy	Zaalag Sabkhas	Duraz Aeolian Sands	Awali Soil with stone pavement	Sakhir Areas of little or no soil Naturally
Geological Mapping Unit	Holocene and Pleistocene	Holocene and Pleistocene sediment	Holocene and Pleistocene sediment	Dammam Group	All horizons
Dominant plant Community	<i>Suaeda</i> spp.	<i>Zygophyllum qatarense</i>	<i>Heliotropium vispium</i>	<i>Zygophyllum qatarense</i>	<i>Zygophyllum qatarense</i>
Soil pH	7.70	8.50	8.00	7.70	7.80
Conductivity (μScm^{-1})	15930	7030	660	2540	2220
TSS (%)	6.70	0.10	0.40	1.12	1.12
Organic Matter (%)	18.34	13.82	1.04	5.14	1.90

Table 1. Sampling sites characteristics and soil analyses.

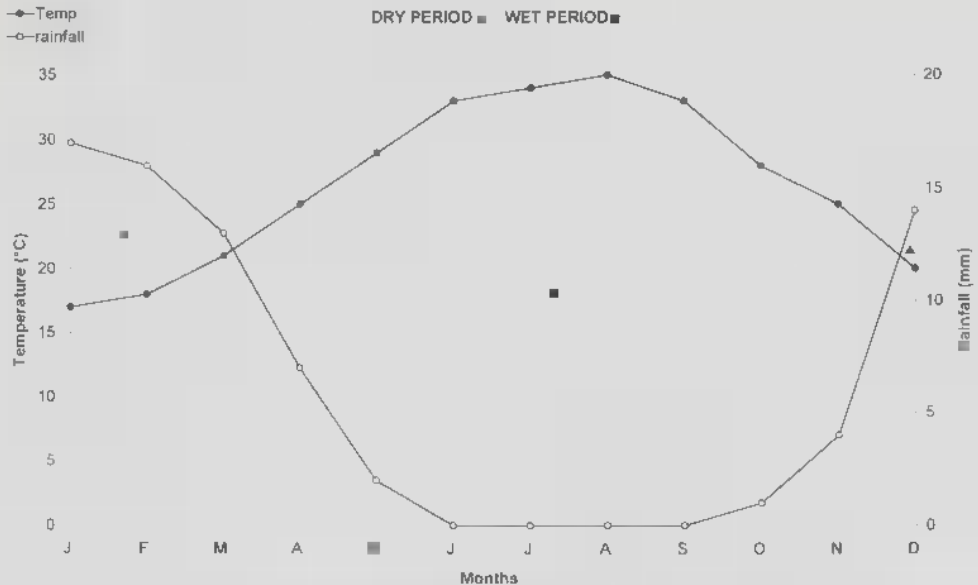


Figure 2. Average monthly rainfall and temperature in Bahrain.

Fusarium species from these composite soil samples: soil dilution, direct soil plating and the baiting technique on selective media.

In the dilution plate method, ten grams of soil are suspended in 90 ml sterile distilled water and thoroughly mixed for 5 min. Soil suspension is allowed to settle for 30 sec and a 1 ml aliquot of the 1:10 dilution is then dispensed uniformly over the surface of six replicate plates per selected medium. Preliminary studies indicated this dilution ratio was the most suitable for samples taken from the various soil groups (15-20 *Fusarium* colonies/plate).

The direct plating technique was modified from McMullen & Stack (1982). For each composite sample 0.05 g was evenly distributed over the surface of a solidified medium. This weight of soil gave similar numbers of *Fusarium* colonies per plate as before (Kreutzer, 1972).

For the baiting technique, young stems and leaves of bean (*Phaseolus vulgaris*) were removed, washed thoroughly in sterilized distilled water, damp dried and then cut into 1 mm pieces. Plant parts were mixed whilst still moist and placed in glass Petri dishes before being autoclaved at 120°C for 15 min; these were then air-dried for 4 hrs. A previously sieved sample from each soil group was hand-mixed with the autoclaved plant parts at a 10:1 ratio. Moisture content of the mixed sample was adjusted to 15% (w/w) and placed in 1 l Erlenmeyer flasks. The latter were shaken thrice weekly and incubated for 21 days at room temperature under continuous cool white lights. Mixed soil samples were then air-dried and again sieved as previously described. Material retained on the 0.5 mm soil screen was further washed for 20 min under tap water (Burgess *et al.*, 1988). Washed particles were then plotted dry for 48 hrs and 0.05g of dried material (for each composite soil) was uniformly sprinkled over the surface of a selected media in six replicates. The

sieved soil of each soil group was treated using the dilution plate technique as previously described.

Isolation plates were incubated at $22 \pm 2^\circ \text{C}$ for 10 days under a 12 hr photoperiod using cool white fluorescent lights (Burgess & Summerell, 1992). Developing colonies on each medium that were putative *Fusarium* species were transferred individually to standard 2% acidified potato dextrose agar medium (APDA) and incubated as described above.

Media used for isolation

The following media were used: Oxoid commercial acidified potato dextrose agar as a nutrient rich medium, Komada selective medium, Nash-Snyder or peptone PCNB agar and selective *Fusarium* agar. All media were autoclaved (15 min at 120°C) and allowed to cool to 45°C before addition of antibiotics, adjustment of pH or addition of heat-labile ingredients. Fifteen ml of each medium were added to Petri dishes stored in the refrigerator for at least five days before use.

For identification purposes three additional media were used: synthetic nutrient agar, potato sucrose agar and 2% water agar.

Identification

Single spore or hyphal tip cultures of representative *Fusarium* species were prepared and maintained on PDA under the incubating conditions described above for seven days. Identifications were made according to Brayford (1993).

Data analyses

To quantify fungal abundance and distribution among soil groups and according to the isolation technique, relative densities were determined. The relative density (%) of a species is defined as the number of its isolates divided by the total number of *Fusarium* isolates.

To estimate the recovery of *Fusarium* species according to the isolation techniques from each soil type, percentage recoveries were determined. Percentage recovery is defined as the number of *Fusarium* species recovered from each soil type, divided by the total number of fungi recovered from the same type.

Chi-square tests were used to test homogeneity in contingency tables prepared using frequencies of occurrence, measure the independence of species recovery by each isolation technique among soil types.

RESULTS

Habitats sampling and soil analyses

Characteristics of the sampling sites and data from soil analyses are listed in Table 1. Samples collected mainly from the central-southern part of the main island (Fig. 1) represent the five major soil group types. Sampling sites located in the physiographic zone of coastal lowlands and the plant cover were mainly dominated by salt tolerant plants, e.g. *Zygophyllum qatarense* and *Heliotropium crispum* (Abbas & El-Qqlah, 1992).

Data extracted from the analyses of the several soil types proved to vary

considerably (Table 1). The highest pH value (8.5) was recorded in natural solonchak soil and the lowest pH (7.7) in soils of cultivated solonchak and raw mineral ones. Electrical conductivity (salinity) fluctuated greatly ranging from as low as $660 \mu S cm^{-1}$ in regosols to as high as $15930 \mu S cm^{-1}$ in cultivated solonchak. The highest total soluble salts were recorded in solonchak soils (6.7%), whereas the lowest was found in raw mineral and rock-dominated areas (1.12%). Organic matter content varied from 18.34% in cultivated solonchak to 1.04% in regosols. Texture of the collected soil samples proved to be generally sandy to sandy loam and gravelly.

Fusarium species prevalence and distribution

Six *Fusarium* species were isolated from the various soil groups. These are listed according to their sections following Brayford (1993): *Fusarium solani* (Mart.) Sacc. (sections *Martiella* and *Ventricosum*), *F. sambucinum* Fuckel (section *Discolor*), *F. compactum* (Wollenw.) Gordon and *F. equiseti* (Corda) Sacc. sensu Gordon (section *Gibbosum*), *F. chlamyosporum* Wollenw. & Reinking and *F. sporotrichioides* Sherb. (section *Arthrosporiella*). The recovery of *Fusarium* species from soil groups considered using different media selective for the genus and three different isolation techniques, are shown in Tables 2 & 3. In general, highest species recovery was from samples from regosols (6 species), followed by natural solonchak (4 species), raw mineral and rock dominated ones (3 species). No *Fusarium* was recovered from cultivated solonchak samples.

The highest average number of isolates was also obtained from regosols (122) as compared to other soil groups. However the mean percentage recovery of *Fusarium*

<i>Fusarium</i> spp.	Soil Group*					RD%**
	CS	NS	R	RM	RD	
<i>F. solani</i>			23 (3)	3		28.26 10.2
<i>F. compactum</i>			25 (23)		2	29.34 46.93
<i>F. sambucinum</i>			8			8.7
<i>F. equiseti</i>			22 (19)		3	27.17 38.77
<i>F. chlamyosporum</i>			4			4.34
<i>F. sporotrichioides</i>		(2)	2			4.08 2.17
Total number of fungi	10	2	326	17	126	

* Soil groups are : CS= Cultivated Solonchak; NS=Natural Solonchak; R=Regosols; RM=Raw Mineral Soils; RD=Rock Dominated Areas.

**Relative Density Values.

Table 2. Occurrence of *Fusarium* species in various soil groups during January 1995 and 1996, isolated using the soil dilution plate method and direct soil plating. Soil plating values are shown between parentheses.

species and respective isolates, in relation to the average total number of other fungi, was highest in natural solonchak composite samples (85.71%), followed by raw minerals (41.6%) and regosols (30.9%) while being lowest in soils of rock dominated area (8.27%). The average relative density of *Fusarium* taxa among the several soil types ranged from 37.74 to 2.17% for *F. solani* and *F. sporotrichioides*, respectively (Tables 2 & 3).

Effect of the isolation technique ■ the recovery of *Fusarium* species

Three techniques were used to compare species recovery from treated soil groups (Tables 2 & 3). A total of 428 *Fusarium* strains were recovered using the soil dilution, soil baiting and direct soil plating techniques. Now recovery of *Fusarium* isolates proved to be highest in the case of regosols using the dilution plate technique (84 isolates), followed by the soil baiting technique (direct soil plating: 65 isolates). Also and except for raw mineral soils, maximum isolate recovery was obtained by direct soil plating as compared to soil dilution using the baiting technique (Table 3). But species diversity varied considerably among soil groups and isolation techniques with some taxa not being recovered from certain soils. The three most frequent *Fusaria* observed were *F. solani*, *F. compactum* and *F. equiseti*. The number of species recovered by soil dilution proved to be greater in the case of regosols in comparison to other soil groups or the two other techniques (Tables 2 & 3).

The highest frequency of occurrence was displayed by *F. compactum* and *F. equiseti* following the soil-baiting technique (80%); *F. solani* stands after when applying

Taxon	Isolation Technique**			
	Soil Dilution	Soil Plating	Soil Baiting	
			Dilution	Plate
<i>F. solani</i>	4.64	12.24	38.14	3.51
<i>F. compactum</i>	0.02	5.85	15.67	4.14
<i>F. sambucinum</i>	23.13***	0.91	2.39	3.02
<i>F. equiseti</i>	0.21	3.89	9.28	1.54
<i>F. chlamydosporum</i>	5.78	2.56	1.75	2.27
<i>F. sporotrichioides</i>	5.94	0.22	0.58	0.75

* Data represent Chi-square statistic at 148.43 (15 d.f.)

** See the Material and Methods section for details on isolation techniques.

*** Values in italics represent source of significant contribution to the chi-squar value.

Table 3. Occurrence of *Fusarium* species in various soil groups during January 1995 and 1996, isolated using the soil baiting method and plated by soil dilution plate method and direct soil plating. Soil plating values are shown between parentheses.

<i>Fusarium spp.</i>	Soil Group *					
	CS	NS	R	RM	RD	RD%**
<i>F. solani</i>			61 (37)	36 (8)	2 (9)	79.2 33.33
<i>F. compactum</i>		3 (12)	1 (15)	1 (22)	1 (11)	9.6 37.03
<i>F. equiseti</i>		2 (16)	2 (13)	10 (12)	3 (7)	11.2 29.62
Total number of fungi	30	40	510	221	297	

* Soil groups are : CS= Cultivated Solonchak; NS=Natural Solonchak; R=Regosols; RM=Raw Mineral Soils; RD=Rock Dominated Areas.

**Relative Density Values.

Table 4. Contingency table* for *Fusarium* species by isolation technique interactions among all soil groups.

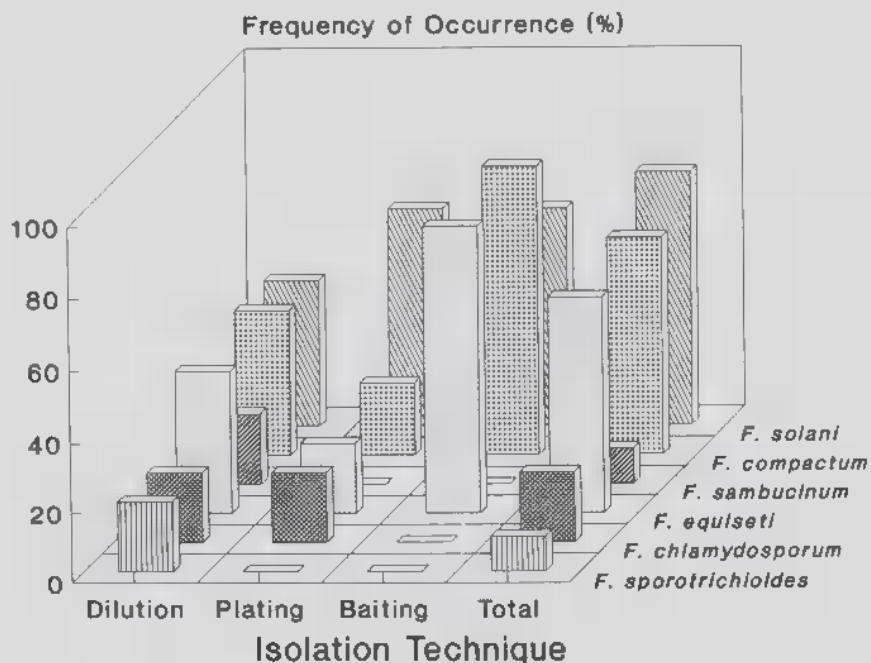


Figure 3. Frequency of occurrence (%) of *Fusarium* species using various isolation techniques.

either the soil plating or the soil baiting techniques (60%) (Fig. 3). The frequency of occurrence of other *Fusaria* ranged from 0-40%. The *Fusarium solani* exhibits the highest total frequency (70%) while *F. sambucinum* and *F. sporotrichioides* displays the lowest ones (10%). *Fusaria* sampled from the different soil types were not recovered independently of the isolation technique applied. Interaction between species and techniques among soil groups proved to be highly significant, as determined by the chi-square contingency table at $P = 0.05$ (Table 4). A greater than expected recovery of *F. sambucinum* and *F. solani* occurred using the soil dilution and the soil plating techniques, respectively. For the soil baiting method (using the soil dilution one), recovery of *F. solani*, *F. compactum* and *F. equiseti* was greater than expected. Similar significant chi-square values were obtained in the contingency table using relative density figures.

DISCUSSION

All *Fusaria* isolated during this study were recorded earlier in cultivated and noncultivated habitats of Bahrain with the exception of *F. sporotrichioides* (Mandeel & Abbas, 1994; Mandeel *et al.*, 1995; Abbas & Mandeel, 1995). In this and in previous studies, the three most frequently recovered species were *F. solani*, *F. compactum* and *F. equiseti* (Fig. 3). These taxa were also reported from desert and saline soils of related Arabian peninsula countries (Mazen *et al.*, 1980; Abdel-Kader *et al.*, 1983; Moustafa & Khosrawi, 1983; Abdel-Hafez *et al.*, 1990; Hashem, 1995). Similar findings were noted from other deserts of the world: French Sahara (Nicot, 1960); Sonaran desert (Ranzoni, 1968); Nevada desert (Durrell & Shields, 1960) and in desert soils of Wadi Bir-El-Ain, Eastern desert of Egypt (Moubasher *et al.*, 1985).

The above *Fusaria* are typically soil-borne fungi persisting in soil in the absence of suitable substrate as dormant chlamydospores (Nash *et al.*, 1961), as resistant hyphae (Nyvall & Kommedahl, 1968) or as conidia in the case of plant residues (Kreutzer, 1972). In terms of host specificity, these *Fusaria* range from highly competitive saprophytes able to interact with other soil microflora (Nyvall & Kommedahl, 1970) to specialized forms attacking several plant families where they induce considerable economic losses (Burgess, 1981). Nutritionally, they are active under a broad range of substrates with mutation being quite frequent within populations to adapt to specific requirements (Toussoun & Nelson, 1975). Also once they are established within a soil niche or as plant pathogens, they are difficult to eliminate.

Species spectrum and numbers can be related to variations in climatic conditions on a large geographical scale basis (Burgess *et al.*, 1988; Kommedahl *et al.*, 1988; Jeschke *et al.*, 1990; Burgess & Summerelle, 1992; Sanglang *et al.*, 1995). In this respect, *F. solani*, *F. compactum* and *F. equiseti* are cosmopolitan in distribution, adapted to a wide range of environmental conditions and habitats, in particular habitats of arid and semi arid regions. Other species (*F. sambucinum* and *F. sporotrichioides*) with specific climatic requirements are restricted to cold temperate and alpine soils; they thus occur in low frequencies in zones unfavorable for their survival. By analogy, the former category may be classified as soil-inhabitants and the latter as soil-transients. However, on a narrow geographical scale basis, *Fusarium* recovery and distribution is also largely influenced by soil factors. Thus, the highest species diversity and isolate recovery occurred in the case of regosols (Tables 2 & 3), probably due to their low salinity levels ($660 \mu\text{Scm}^{-1}$), as compared to other soil types. Similar findings were reported elsewhere (Khodair *et al.*, 1991; Abbas & Mandeel, 1995).

Although it is difficult to relate variations in *Fusarium* population and spectra to a specific soil factor, the exerted effect of high salinity levels counteracted richness in organic matter as a food source and thus greatly reduced *Fusarium* recovery from the same soils (Table 1). Therefore, *Fusarium* were not recovered from soil types rich in organic matter, such as cultivated solanchak (18.34%) having ■ high total soluble salts (6.7%), presumably because of their high electrical conductivity: 15930 $\mu\text{S cm}^{-1}$ (Tables 1, 2 & 3). Recovery of *Fusarium* species increased in soils of raw minerals characterized by low salinity levels, low to moderate organic matter content and soluble salts, followed by natural solonchak soils. Similar observations were also reported by Abdel Fattah *et al.*, (1977) from salt marsh soils. Variations in pH does not seem to affect species prevalence and density except possibly in the extremes (Mandeel & Abbas, 1994).

Low recovery levels and limited species variability was found in noncultivated soil types as compared to cultivated soils (Mandeel *et al.*: 1995). This is in agreement with other findings (Nash & Christou, 1965). Stoner (1981) stated however that "since the roles of these fungi (*Fusarium*) are not restricted to parasitism and disease and can involve nutrient cycling and other functions in noncultivated soils, it is conceivable that large populations are not a prerequisite to significant ecological impact".

In noncultivated hot desert ecosystem, where *Fusarium* species are present at discrete locations in low frequencies and in various forms, it becomes essential to use many isolation techniques to adequately demonstrate species abundance and distribution (Stoner, 1981; McMullen & Stack, 1982). Based on modes of persistence in soil, isolation technique can greatly affect rate of recovery of individual members within the genus. Because soils in Bahrain are formed from Holocene and Pleistocene sedimentary rocks that are saline and generally poor in organic matter (Table 1), isolation methods applied here aimed to isolate species from these groups of soils. The soil dilution technique decreases soil salinity by 10% and favours the isolation of species sporulating profusely or which are basically chlamydopores formers (Burgess & Summerell, 1992). Although the technique has some limitations, it reflects the general distribution trends associated with microbial activity and nutrient cycling processes, especially in stressful environments such as deserts (Mandeel *et al.*, 1995). For fast growing species persisting in soils as mycelium or by adherence to humus or mineral particles, the soil plating technique is appropriate. The baiting procedure was designed to recover competitive saprophytes by the addition of an organic substrate. In the present work, highest species diversity and density were found with the soil dilution method from samples of regosols as compared to the other methods (Tables 1 & 2).

Although certain species were not recovered by one or the other technique, it is difficult to assess whether recovery of an individual species was favoured or inhibited by the isolation technique. In addition, Sanglang *et al.* (1995) indicate that inability to recover a species from a particular soil in a climatic region do not imply the fungus is totally absent even when present at low frequencies, given the nature of the isolation technique used. Therefore, for quantitative and qualitative comparisons of soil *Fusarial*, several techniques must be used to avoid any possible bias in the survey results (Burgess, 1981; McMullen & Stack, 1982).

Analysis of the relationships between species recovery and isolation technique as determined by chi-square tests revealed significant interactions (Table 4). These suggest some techniques allow a greater recovery of *Fusarium* species from the different soil groups. On the whole, soil dilution or plating using the baiting technique yielded higher isolate numbers for *F. solani*, *F. compactum* and *F. equesiti* from all soil groups as compared to other techniques (Tables 1, 2).

Mandeel *et al.* (1995) compared several media for *Fusarium* recovery from cultivated and noncultivated habitats of Bahrain. They concluded that standardization of isolation media greatly minimizes variations within population types. This is particularly important when comparing surveys from arid habitats. Ideally, more than one technique and media should be used for recovery of soil-borne *Fusarium* species.

Several investigators did not disclose significant differences in the density or diversity of *Fusarium* species isolated by soil dilution, soil plating or debris plating techniques (McMullen & Stack 1982). But when comparisons were made for soils of different altitudes, the diversity of *Fusarium* species was found to be greater when using the debris plating technique rather than the soil dilution plating (Jeschke *et al.*, 1990). In the present study, use of the soil dilution technique gave a recovery of *Fusarium* species by lowering salinity levels (Table 1).

The results of the present work show that observed spectra and densities of *Fusarium* species are low when compared to data for cultivated soils. Also predominant species appear better adapted to extreme environment and have broader nutrient requirements; such accounts for their higher frequency when compared to other species. Further, since each isolation technique and medium used is somewhat selective, it is difficult to recover all species by one technique and one medium. It is therefore important to use a combination of various isolation methods and media to obtain good estimates of a populations density and the diversity of species within the genus in case of soil surveys.

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